HYDROGEN-ION CONCENTRATION OF PROTOZOAN CULTURES.

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The chemical changes taking place in ordinary hay infusions used for protozoan cultures have been described by various authors, among whom may be mentioned Peters[1] and Fine.[2] The points of interest discussed by these two authors are the changes occurring in the titratable acidity and alkalinity of such infusions. Changes in titratable acidity and alkalinity, however, as is well known, do not give a correct expression of the changes in concentration of hydrogen ions. Inasmuch as the hydrogen-ion concentration of the medium with which organisms are in contact is of great physiological importance, it was planned to study this factor throughout the life of series of protozoan cultures, and to see in how far such results agreed with those obtained by titration methods.

In the present study, therefore, determinations were made of both titratable acidity and alkalinity, as well as of hydrogen-ion concentration of series of cultures prepared in various ways.

The titratable acidity was obtained by titrating 5 c.c. of culture with 0.01 *N* NAOH, using phenolphthalein as indicator. The titratable alkalinity was obtained by titrating 5 c.c. of culture with 0.01 *N* H_2SO_4, using bromphenol blue as indicator. Hydrogen-ion determinations were made by colorimetric methods, using phosphate and acetate mixtures as standards, and phenol-sulfonephthalein, brom-cresol purple and methyl red as indicators. Series of cultures were prepared in various ways and determinations carried out daily. In the case of hay infusion cultures with hay, the same amount of hay (approx. 25 gms.) was used and treated in the manner indicated in explanation of figures. The soil in soil cultures was obtained from the greenhouse and was

[1] Peters, A. W., 1907, *Amer. Jour. Physiol.*, 17 and 18.

[2] Fine, M. S., 1912, *Jour. Exp. Zoöl.*, 12, p. 265.

that used by the gardener in ordinary greenhouse work. Total volume of cultures varied from 2 to 5 liters, depending on size of container used. When cultures were inoculated with organisms samples were taken from several cultures so that as many representative protozoan forms as possible were obtained. All cultures were kept under the usual laboratory conditions, in the same room, with the temperature as nearly constant as possible.

The data obtained in such a study are most readily presented by curves, since it is relative and not absolute changes in which we are interested.

Peters[1] and Fine[2] found that the top and bottom of cultures varied in titratable acidity—at first, a high titratable acidity at the bottom, and later, as diffusion proceeded, a more or less uniform concentration was obtained and titratable acidity differences tended to disappear. A similar condition has been found by the author in titrations, but the differences in hydrogen-ion concentration are never great and, when occurring, last only for a very short time in the early life of the culture. In the following results, however, average conditions only will be given.

Figure 1 shows graphically the changes in Ph in a typical soil culture. These cultures, during the first few days, become slightly acid, followed by a less acid condition, and, finally, remain rather alkaline throughout the remainder of the experiment. Observation showed that organisms were most abundant during the early life of the culture, when the change from a slightly acid to a slightly alkaline reaction was taking place.

Figure 1 also shows the changes in Ph in hay infusion cultures prepared in slightly different ways. From these results it is quite evident that the presence of hay, either boiled or unboiled, tends to make a decided difference in the hydrogen-ion concentration of the culture. No attempt was made to keep the cultures sterile, and since it has been pointed out repeatedly that the bacteria are to a great extent, if not entirely, responsible for the acid production, these changes in Ph between cultures with and without hay are in all probability due to amounts of available food for bacterial organisms, as well as to the acid-yielding con-

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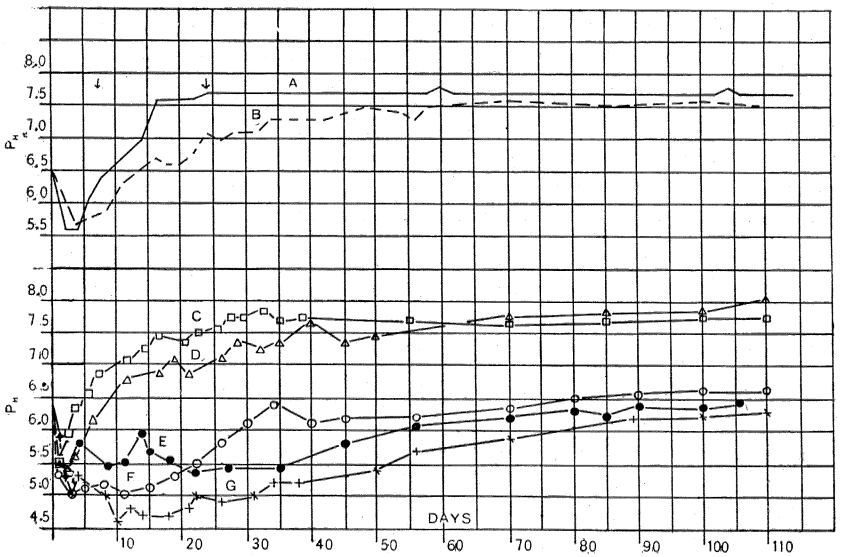

FIG. 1. *Top*—Curves show the Ph of soil and ordinary hay infusion cultures for a period of over 3 months.

A = soil culture, prepared with soil, hay infusion, tap water and inoculated with organisms.

B = culture prepared with hay infusion, tap water and inoculated with organisms.

Space between arrows indicates time when organisms appeared to be most abundant.

Bottom—Curves show the Ph of cultures with and without hay and also the Ph of inoculated and non-inoculated cultures.

C = culture prepared with hay infusion, tap water and inoculated with organisms.

D = culture prepared with filtered hay infusion and tap water and not inoculated.

E = culture prepared with hay, filtered hay infusion and tap water and not inoculated.

F = culture prepared with boiled hay, hay infusion, tap water and inoculated with organisms.

G = culture prepared with boiled hay, filtered hay infusion and tap water and not inoculated.

Volume of culture, 5 liters. Abscissas, time in days indicated. Ordinates represent Ph.

stituents of the hay. The differences in Ph between inoculated and non-inoculated cultures are probably due, either to a reduction of the bacteria by the protozoa, or possibly to various excretion products of the protozoa themselves.

It is also of interest to point out here that in the soil cultures there is a relatively short period of acidity followed by a longer

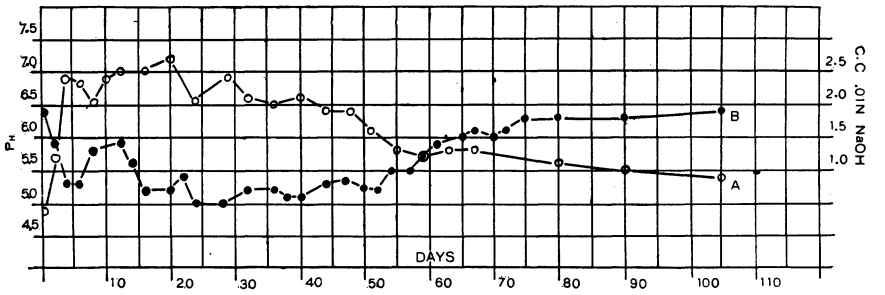

FIG. 2. Curves show the titratable acidity and Ph of an ordinary hay infusion culture, prepared with boiled hay, filtered hay infusion and tap water. *A* = titratable acidity. *B* = Ph. Abscissas time in days indicated. Ordinates at left represent Ph. Ordinates at right represent cubic centimeters 0.01 *N* NAOH required to neutralize 5 c.c. of culture.

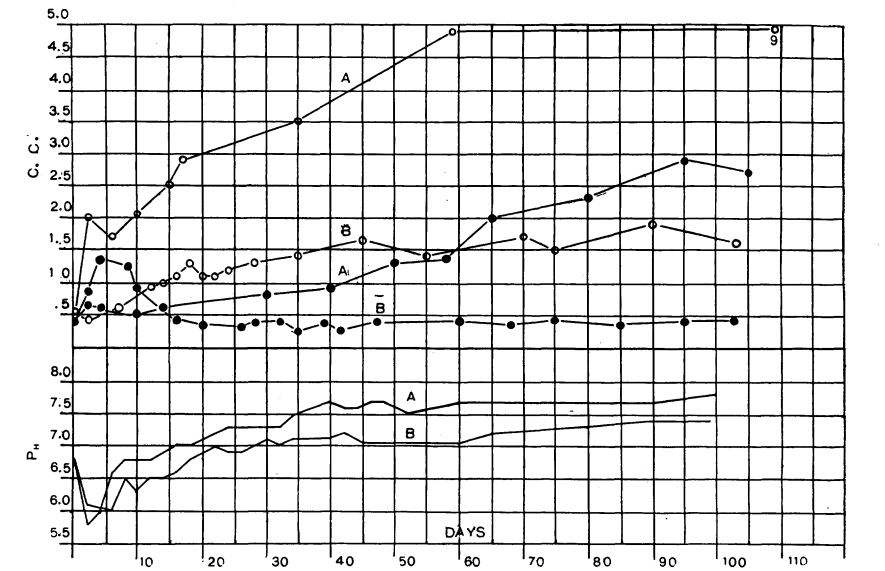

FIG. 3. Curves show the titratable acidity and alkalinity and the Ph of an ordinary hay infusion culture and of a hay infusion culture to which a piece of marble has been added. *A* = culture prepared with boiled hay, hay infusion, tap water, piece of marble and inoculated with organisms. *B* = culture prepared with hay infusion, tap water and inoculated with organisms. Volume of cultures, 5 liters.

Top—Curves show titratable acidity and alkalinity. O = alkalinity. ● = acidity. Abscissas, time in days indicated. Ordinates represent cubic centimeters 0.01 *N* NAOH and H_2SO_4 to neutralize 5 c.c. of culture.

Bottom—Curves show Ph of cultures. Abscissas, time in days indicated. Ordinates represent Ph.

one of alkalinity, while in hay cultures there is a period of acidity which lasts for a relatively long time in the life of the culture.

Figure 2 shows graphically the relation between the titratable acidity and hydrogen-ion concentration of a typical hay infusion culture. A somewhat more gradual and noticeable change is noted for titratable acidity than for hydrogen-ion concentration, and this seems to show that a marked change in titratable acidity is not necessarily accompanied by a correspondingly marked change in hydrogen-ion concentration.

Peters[1] and Fine[2] have shown that the acidity of hay infusions is due almost entirely to bacteria, to carbon dioxide, and acid-yielding hay constituents. This being the case, it is of interest to see how in an ordinary hay infusion a piece of marble will produce a fairly constant Ph.

Figure 3 shows graphically the changes in Ph and in titratable acidity and alkalinity in such a culture. These cultures gradually become alkaline in reaction, as indicated by Ph, but still have an appreciable titratable acidity. Cultures prepared in such a manner ought to be useful for keeping organisms in media of almost constant Ph.

The above results on titratable acidity and alkalinity are quite in agreement with those reported by Peters[1] and Fine,[2] and in addition show the relative changes in hydrogen-ion concentration during the life of hay infusion and soil cultures. The sequence of organisms and changes in hydrogen-ion concentration have not been particularly studied, but it is not improbable that some relation between the two might be shown to exist.

[1] Peters, A. W., 1907, *Amer. Jour. Physiol.*, 17 and 18.

[2] Fine, M. S., 1912, *Jour. Exp. Zoöl.*, 12, p. 265.